

Mutants-P318A.ST25
SEQUENCE LISTING

<110> Covalys Biosciences AG

<120> Mutants of O6-Alkylguanine-DNA Alkyltransferase

<130> P318A

<150> EP04405123.3

<151> 2004-03-02

<150> EP04405465.8

<151> 2004-07-22

<160> 48

<170> PatentIn version 3.3

<210> 1

<211> 624

<212> DNA

<213> Homo sapiens

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gctgatgccg tggaggtccc agcccccgct gcggttctcg	gaggtccgga gcccctgatg	180
cagtgcacag cctggctgaa tgcctatttc caccagcccg	aggctatcga agagttcccc	240
gtgccggcac ttaccatcc cgttttccag caagagtcgt	tcaccagaca ggtgttatgg	300
aagctgctga aggttgtgaa attcggagaa gtgatttctt	accagcaatt agcagccctg	360
gcaggcaacc ccaaagccgc gcgagcagtg ggaggagcaa	tgagaggcaa tcctgtcccc	420
atcctcatcc cgtgccacag agtgggtctgc agcagcggag	ccgtgggcaa ctactccgga	480
ggactggccg tgaaggaatg gcttctggcc catgaaggcc	accggttggg gaagccaggc	540
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<211> 33

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<220>
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<210> 4
 <211> 43
 <212> DNA
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<210> 5
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 gcaaccccg c agccacggca gcagtgggag g 31

<210> 6
 <211> 31
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<220>
 <223> Antisense primer for mutating K125A, T127A, R128A
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<210> 7
 <211> 49
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> sense primer for cloning into eukaryotic pNUC vector
 <400> 7
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<210> 8
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning into eukaryotic pNUC vector
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<210> 9
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<220>
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<210> 10
 <211> 35
 <212> DNA
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<220>
 <223> Antisense primer for mutating Cys 62 to Ala
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 gcattcagcc aggctgtagc ctgcatcagg ggctc 35

<210> 11
 <211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Sense primer for cloning of AGT mutants into phage-display vector
 <400> 11
 ctactcgcgg cccagccggc catggcggac tacaaagaca tggacaagga ttgtgaaatg 60

<210> 12
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning of AGT mutants into phage-display vector
 <400> 12
 ggaattcggc ccccgaggcc gcgtttcggc cagcaggcgg 40

<210> 13
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense primer for cloning AGT truncated after 182 into pGEX
 <400> 13
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<210> 14
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 <223> n is a, c, g, or t

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 <223> n is a, c, g, or t

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 <223> n is a, c, g, or t

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 <223> n is a, c, g, or t

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 <210> 15
 <211> 26
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense primer for randomisation of codons 131-135

 <400> 15
 tgctcgcgcg gctttggggg tgcctg 26

 <210> 16
 <211> 41
 <212> DNA
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 <400> 16
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 <210> 17
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 <223> Antisense primer for randomisation of codons 115-116

 <400> 17
 gtaagaaatc acttctccga atttcac 27

 <210> 18
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 <212> DNA
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<220>
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<210> 19
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 <212> DNA
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<220>
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<400> 19
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<210> 20
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<220>
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<400> 20
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<210> 21
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 <212> DNA
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<220>
 <223> Antisense primer for mutating G131K, G132T, M134L, R135S

<400> 21
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<210> 22
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<220>
 <223> Sense primer for mutating Q115S, Q116H

<400> 22
 gtgaaattcg gagaagtgat ttcttactct cacttagcag c 41

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<210> 23
 <211> 35
 <212> DNA
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 <223> Antisense primer for mutating Q115S, Q116H

 <400> 23
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<210> 24
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 <223> Sense primer for mutating C150N, S151I, S152N

 <400> 24
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 <400> 25
 cgtaaccgcc cacggctcca ttgatattga cc 32

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<210> 27
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 <223> Primer for cloning mutant AGT in pET15b

 <400> 27
 gattacggga tccttatccc aagcctggct tccc 34

<210> 28
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 <220>
 <223> Antisense primer for cloning truncated AGT in pAK 100

 <400> 28
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<210> 29
 <211> 81
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 <220>
 <223> Sense primer for introducing 12xHis, SbfI and AscI sites

 <400> 29
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 ggcgcgcta aaagcttctt a 81

 <210> 30
 <211> 81
 <212> DNA
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 <220>
 <223> Antisense primer for introducing 12xHis, SbfI and AscI sites

 <400> 30
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 atgatgatgt gccatggata a 81

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 <220>
 <223> Sense primer for cloning mutant AGT in pBAD-HisA

 <400> 31
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 <210> 32
 <211> 45
 <212> DNA
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 <223> Antisense primer for cloning mutant AGT in pBAD-HisA

 <400> 32
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 <212> DNA
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 <223> Antisense primer for cloning wt AGT in pBAD-HisA

 <400> 33
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 <210> 34
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 <212> DNA
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<223> Sense primer for cloning AGTM in pEGFP-Nuc

<400> 34
gatcgagcta gcgctaccgg tcgccaccat ggacaaggat tgtgaaatg 49

<210> 35
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<212> DNA
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<223> Antisense primer for cloning AGT G160W in pEGFP-Nuc

<400> 35
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<210> 36
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<212> DNA
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<223> Antisense primer for cloning AGTM in pEGFP-Nuc

<400> 36
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<210> 37
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<210> 38
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<400> 38
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<210> 39
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<223> Sense primer for mutation G160W

<400> 39
caactactcc tggggactgg ccgtg 25

<210> 40
<211> 25
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 <210> 41
 <211> 39
 <212> DNA
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 <223> Primer for error prone PCR of pAK100 insert, c at position 1
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 <211> 39
 <212> DNA
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 <223> Antisense primer for error prone PCR of pAK100 insert
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 <210> 43
 <211> 19
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Sense primer for amplification of errorprone-PCR product
 <400> 43
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 <210> 44
 <211> 19
 <212> DNA
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 <223> Antisense primer for amplification of errorprone-PCR product
 <400> 44
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 <210> 45
 <211> 48
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 <223> Sense primer for saturation mutagenesis AGTM 150-154

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<400> 46
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48

<210> 47
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